UV optical difference spectrum associated with the reduction of electron acceptor A_1 in photosystem I of higher plants

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Photosystem I particles containing 1 P700 per 32 chlorophyll molecules were illuminated at cryogenic temperatures in the presence of sodium dithionite. Under conditions which specifically led to reduction of acceptor A_1 (as shown by its characteristic EPR spectrum) optical absorbance changes were detected between 240 and 325 nm. The appearance of these changes correlated closely with the increase in amplitude of the A_1 EPR signal. The possibility that a quinone-like species is associated with, or directly involved in intermediary PS I electron flow is discussed.

Photosystem I   *Electron acceptor*   *Ultraviolet optical difference spectrum*   *Quinone*

1. INTRODUCTION

The nature of the electron-accepting system of PS I has been investigated by, amongst other methods, progressive reduction of its component parts and subsequent observation of optical and EPR spectra. The secondary acceptors, iron-sulphur centres A and B (E_m −0.55 and −0.59 V respectively [1,2]), may be reduced chemically. An intermediary acceptor, centre X (E_m −0.70 V [3]) may be only partially reduced chemically and can be fully reduced by illumination at low redox potential. Under these conditions optical and EPR spectra have indicated that X is also an iron-sulphur component [4,5]. Flash and continuous illumination of chemically reduced samples at room and cryogenic temperatures led to the discovery of a lower potential acceptor complex 'A_1' [4,6,7]. This was subsequently resolved into two distinct components: 'A_1' and an earlier acceptor 'A_0' [8,9]. The EPR signal associated with A_0 is

Abbreviations: Chl, chlorophyll; PS, photosystem; UV, ultraviolet

characteristic of a chlorophyll monomer. Previously we reported optical changes that accompanied reduction of A_0 [10]. A difference spectrum in the visible region had minima at 670, 430 and 405 nm and a maximum at 450 nm. The amplitude of the 670 nm change correlated well with that of the $g = 2.0$ EPR spectrum associated with A_0 and we concluded that the two were derived from the same component. From the shape of the optical signal we proposed that A_0^- is a Chl *a* anion monomer [10].

Upon reduction of A_1 it has not proved possible to detect any optical absorbance changes in the visible region [10,11]. A_1^- is thought to back-react with P700$^+$ with a $t_{1/2}$ of 120 μs at 10 K as measured by relaxation of the 820 nm absorption associated with P700 [11]. However, in the range 650–730 nm all absorbance changes could be accounted for by P700$^+$ re-reduction, with no contribution by A_1^- oxidation. The absence of optical changes in the visible region reported in the above experiments indicates that A_1 is not a chlorophyll or a pheophytin molecule. A_1 has an asymmetric X-band EPR spectrum [8] and in this respect

resembles certain bound quinone species; examples are given in [12,13]. Moreover, at higher, Q-band microwave frequencies the A_1^- spectrum has 'g_{xx}' and 'g_{yy}' values of 2.0055 and 2.0046, respectively. These values are typical of a randomly orientated radical with g anisotropy [14]. The values are slightly less than, but comparable to, values derived from a deuterated benzosemiquinone anion radical in organic solution [12] and from Q_a^- in deutered bacterial reaction centres [15]. These indications that A_1 resembles a quinolic species led us to investigate its optical properties in the UV region.

Here we show that absorbance changes can be detected between 240 and 325 nm during progressive photo-reduction of A_1. The amplitude of these signals correlates well with the trapping of A_1 in the reduced state as detected by EPR measurements. The optical difference spectrum obtained has similarities with published difference spectra for reduction of some quinone species to the semiquinone form.

2. MATERIALS AND METHODS

PS I particles were prepared from pea (*Pisum sativum* var. Feltham First) leaves using Triton X-100 digestion and subsequent purification on a hydroxyapatite column [10,16]. The 10 mM potassium phosphate elution from an initial column was re-chromatographed on a second hydroxyapatite column and again eluted with 10 mM phosphate, pH 6.8. The final P700:chl ratio was 1:32, the sample being concentrated using an Amincon XM-100 filter.

The standard assay mixture and sample preparation for both EPR and optical measurements were as in [10]. However, a 2 min room temperature pre-illumination to reduce electron acceptors X, A and B was carried out using light from a Barr and Stroud 150 W fibre optic light source, filtered through a copper sulphate solution. During this process the samples were maintained under a stream of nitrogen either in an EPR tube or in an optical cuvette consisting of two quartz plates separated by a 0.5 mm thick latex rubber gasket and supported in a brass frame. No discolouration of samples occurred during this treatment. After a 10 s dark period the samples were cooled to 205 K

in the cryostat of the appropriate measuring instrument.

Optical measurements were made on a Cary 219 spectrophotometer in the double-beam mode with a 3 nm slit width. The 'sample' carriage was replaced by an Oxford Instruments cryostat which was cooled to the required temperature with a liquid nitrogen supply. No 'reference' sample was used. The sample was held at 45° to the measuring beam to facilitate both measurement and illumination through a side window without moving the cuvette. Spectra were recorded on a Tektronix 4051 computer, taking 1000 data points per 85 nm, scanning at 0.5 nm per s. EPR measurements were carried out and standardised as described in [10]. Illumination of samples in either the EPR or optical cryostats was carried out using the fibre optic light source described earlier.

3. RESULTS AND DISCUSSION

In fig.1, we show an EPR signal recorded in the $g = 2.0$ region that resulted from illumination of PS I particles in the presence of sodium dithionite at 205 K. Spectra were recorded in the dark following illumination for the stated periods. The signal was asymmetric and centred at $g = 2.0053 \pm 0.00057$ and was 1.096 ± 0.105 mT wide (from 8 different samples). In these respects it may be taken to represent progressive reduction of electron acceptor A_1 (these values may be compared to those in [8,9]). At 150 μg/ml the chlorophyll concentration was low compared with usual EPR samples and the signal to noise ratio was correspondingly decreased. This concentration was chosen so that readings would be directly comparable with optical measurements. Each successive spectrum showed no tendency to decay over the ensuing 10 min dark period thus indicating that the acceptor was 'trapped' in the reduced state. Moreover, there appeared to be no lag period in the formation of the A_1 signal, suggesting that centre X was fully reduced. At the end of the illumination regime the sample was warmed to room temperature and re-frozen in the dark. Following this procedure the $g = 2.0053$ signal completely disappeared. This demonstrated that the trapped acceptors were able to relax fully and that the signal was not due to non-specific degradation of the sample.
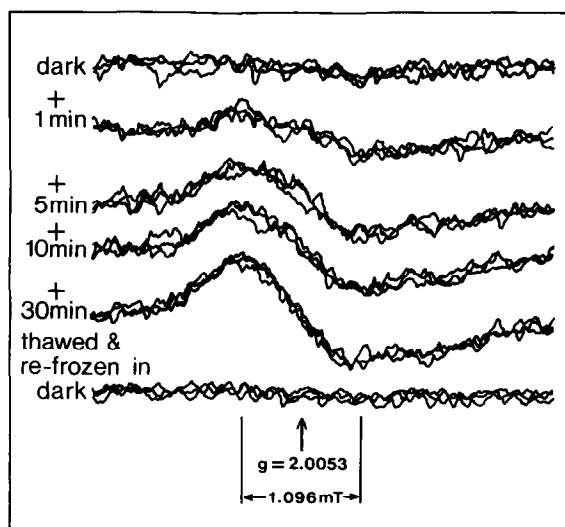
Fig.1. EPR spectra of A_1 recorded after illumination (for the stated time increments) of PS I particles at 205 K. The chlorophyll concentration was 150 μg/ml with 1 P700/32 Chl. The iron-sulphur acceptors (A,B,X) were reduced prior to low-temperature illumination. The spectra were recorded at 205 K with the following instrument settings: microwave power, 100 μW; frequency, 9.036 GHz; modulation amplitude, 2 G; instrument gain, 10^4; response time, 1 s. Each spectrum consists of three superimposed scans recorded in the dark over a period of 10 min during which the signal showed no tendency to decay.

Identical samples were assayed optically in the UV region (240–325 nm). An initial 'dark' spectrum was obtained from the sample prior to low-temperature illumination; five separate scans were averaged to obtain the spectrum. Spectra from six similar samples were then averaged and the results presented in fig.2a. The broad absorbance centred at 312 nm is due entirely to the presence of reduced sodium dithionite. The peak at 278 nm results, in part, from absorbance by Triton X-100. Superimposed upon the dark spectrum is the spectrum recorded at the end of the illumination regime (i.e. after a total of 46 min in the light). At the given scale no clear differences can be seen, except for broadening of the traced line at certain wavelengths. Following recording of the initial dark spectrum, a second set of five scans of the un-illuminated sample were averaged. The difference between these two sets was taken as the 'dark-dark' difference spectrum (fig.2b). It can be seen
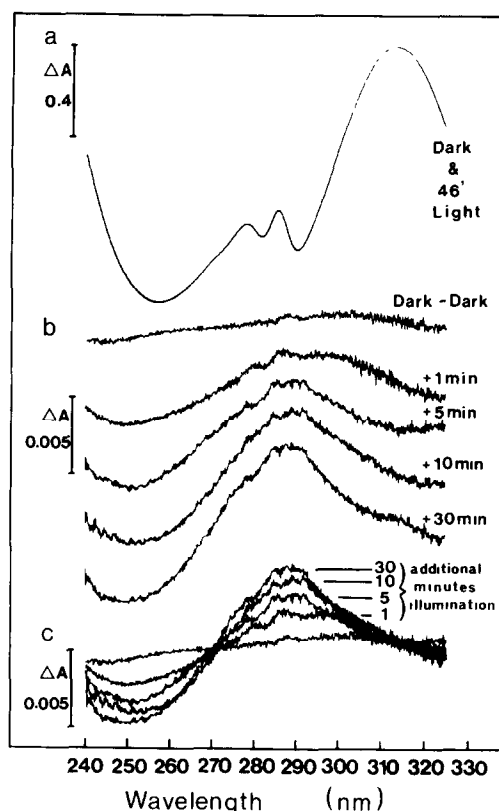

Fig.2. Optical spectra of pea PS I particles measured at 205 K. (a) Absorbance spectra of the sample before and after 46 min illumination at 205 K. (b) Difference spectra recorded in the dark following illumination for the stated time increments. (c) Superimposition of the difference spectra shown in (b). Chlorophyll concentration was 150 μg/ml. Five spectra were recorded and averaged for each time increment on an individual sample. Results from six similar samples were then averaged to give the spectra shown here.

that essentially no absorbance changes had occurred in the dark during these procedures. The dark-dark difference spectrum is not perfectly flat, however, this may be the result of excitation of the sample by the measuring beam. Subsequently, spectra were recorded in the dark following the illumination periods indicated. The initial dark spectrum was subtracted from each subsequent spectrum and the differences are presented (fig.2b). It can be seen that after illumination for 1 min a difference spectrum with both an absorbance increase and a bleaching appears. These dif-

ferences increased upon further illumination giving a maximum at 287 nm and a minimum at 250 nm. There was an isosbestic point at 269 nm, which may be seen more clearly when the difference spectra are superimposed (fig.2c). The amplitude of the 250 nm bleaching changed at a similar rate to the increase in absorbance at 270 nm, indicating that the two are probably derived from the same component. The signal remained stable, apparently indefinitely, in the dark. The affected component therefore was trapped in its altered state. It was not possible to warm the sample in order to check for reversibility since, as noted in [10], the suspension becomes opaque on warming above 220 K. A spectrum was taken from a sample that was allowed to become opaque; the difference between this and the original dark spectrum did not resemble the difference spectra shown in fig.2 (not shown). The recorded spectra, therefore, were not the result of anomalous warming effects.

The peak to trough amplitudes of the EPR signals (fig.1) and the optical difference spectra (fig.2) were expressed as a percentage of the amplitude after the final illumination period and plotted for comparison (fig.3). It can be seen that the rate at which the two signals appear is identical. This indicates strongly that the same
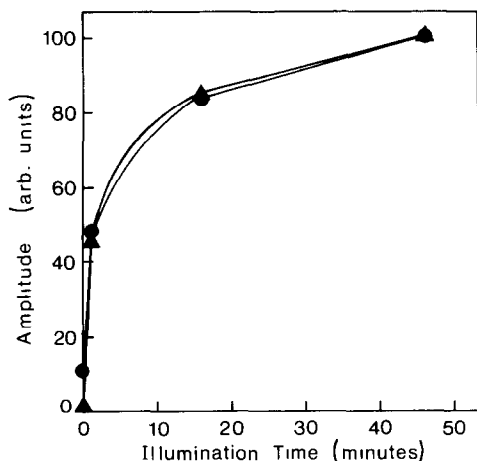

Fig.3. Comparison of the peak-to-trough amplitude of EPR and optical spectral changes following low-temperature illumination of PS I particles. Data were taken from fig.2 and from the average of eight similar samples to that shown in fig.1. (▲——▲) Optical data, (●——●) EPR data.

phenomenon is responsible for each signal. The optical spectrum must derive either from reduction of A_1 itself or from some other component in its environment that is susceptible to the redox state of A_1. It should be noted that both signals approached saturation levels. This suggests that a discrete component having a fixed stoichiometry with the reaction centre was reduced.

We have shown here that photochemical reduction of PS I electron acceptor A_1 is accompanied by optical changes with a minimum at 250 nm, a maximum at 287 nm and an isosbestic point at 269 nm. Such changes are comparable with spectra that have been recorded upon reduction of various species of quinone [17]. The latter spectra were measured in methanolic solution using pulse radiolysis to generate neutral or anionic semiquinones, depending on the prevailing pH. The characteristics of the difference spectrum presented here correspond most closely with the various neutral semiquinone spectra shown in [17]. Absolute semiquinone spectra were determined in [17] and these showed that the absorbance peak for anionic semiquinones occurred at about 320 nm. However, for the protonated neutral semiquinones the peaks were somewhat below 300 nm which corresponds with the maximum absorbance increase (at 287 nm) shown in this work. Additionally, it has been shown that whereas immobilised anionic semiquinones have relatively narrow (0.85 mT) and Gaussian EPR spectra the neutral forms are broader and asymmetric [18] and are therefore similar to the A_1 EPR signal presented here. The presence of quinones in PS I has been well documented [19–22]. Specifically, phylloquinone or vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) preferentially segregates with PS I fractions [19–21]. Recently a stoichiometry of 1 P700/2 phylloquinone molecules has been determined, a result that implicates a functional role for these quinones [23]. However, competitive inhibition experiments have failed to indicate any indirect role in linear electron flow to artificial electron acceptors [24]. Perhaps the major problem with assigning a functional role to a quinone on the acceptor side of PS I is that the electrical potential of such a component would necessarily be very low (in the region of −800 to −900 mV). However, typical midpoint potentials of quinolic redox couples are found in the range 900 to −200 mV at various pH

values [25]. The midpoint potential of the bacterial reaction centre acceptor quinone is -150 mV [26] and in PS II the electron gating mechanism operates at about 0 mV. A bound quinone operating on the acceptor side of PS II has been titrated at E_m -250 mV however [27]. Very low redox potentials are not impossible for quinolic couples; an $E_{1/2}$ of -600 mV has been recorded for a substituted quinone [28] and, as discussed in [22], a suitable aprotic environment may lower this value even further. Indeed, interaction of two neighbouring quinones may influence this process.

It is possible that reduction of electron acceptor A_1 causes an absorbance change of a non-functional quinone in its environment, hence giving the difference spectrum that we have observed. If this were the case however, we would be left to assume that A_1 gives no detectable spectral changes in the range 240–750 nm upon reduction. The proposition that A_1 itself is a quinone leads to various experimental possibilities which we are at present pursuing.

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